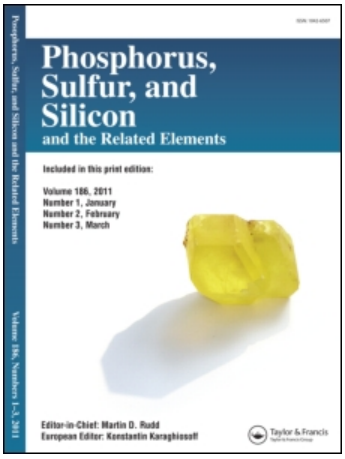

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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To cite this Article Janda, Kim D. and Wentworth, Anita D.(1999) 'A Merging of Chemistry and Biology in Terms of Antibody Catalysis', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 117 — 120
To link to this Article: DOI: 10.1080/10426509908546196
URL: http://dx.doi.org/10.1080/10426509908546196

Phosphorus, Sulfur and Silicon, 1999, Vol. 144-146, pp. 117-120
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A Merging of Chemistry and Biology in Terms of Antibody Catalysis

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Novel hapten design strategies to improve antibody catalysis of aryl ester, glycoside and primary amide bond hydrolyses are discussed.

Keywords: Catalytic antibody; hapten; reactive immunization; chemical selection

INTRODUCTION

The ability of the immune repertoire to elicit high affinity protein receptors (antibodies) to virtually any chemical ligand (hapten) has been utilized to generate programmable biocatalysts with a broad range of applications[1,2]. In accordance with Jencks' prediction[3], the majority of catalytic antibodies reported to date have been raised against transition state analogs (TSAs) for the reactions which they accelerate. Featured in this article are examples of strategies being pioneered by our group to refine hapten design.

Reactive immunization

Kinetic resolution of aryl esters of naproxen

Limitations of the TSA hapten approach are being addressed by modifications in immunogen design to supplement transition state stabilization in antibody combining sites with additional catalytic mechanisms[1,2]. Reactive immunization[4], a method finding increasing utility, employs a 'reactive' hapten that can either hydrolyze slowly at physiological pH to release a conventional TSA, or trap a nucleophile at the B-cell level of the immune response. This strategy was utilized to generate catalysts for the hydrolysis of aryl esters of naproxen **1** to facilitate the kinetic resolution of this chiral anti-inflammatory agent (see Figure 1)[5]. Following immunization of BALB/c mice with a keyhole limpet hemocyanin (KLH) conjugate of the labile phosphonate diester hapten **2** (half-life at pH 7.4 = 48 h), a panel of 20 hapten-specific monoclonal antibodies was generated. All 12

identified catalysts exhibit the correct stereoselectivity to generate only the active enantiomeric acid *S*-(+)-**1** from a racemic mixture of *p*-(methylsulfonyl)phenyl esters *rac*-**3**. The 5 most active antibodies possess some of the highest proficiency quotients ($[(k_{cat}/K_m)/k_{uncat}] > 10^9 M^{-1}$[6]) reported for catalytic antibodies, even comparable with a number of enzymes. Furthermore, their calculated E values of > 20 ($[k_{cat}(S)/K_m(S)]/[k_{cat}(R)/K_m(R)]$) predict enantiomeric excesses (*ee*s) of >98 %[7]. However, the best observed *ee* was only 90 % after 26 % conversion of *rac*-**3**, this inconsistency being attributed to the inhibitory activity of the ester *R*-(-)-**3**. It is postulated that by immunizing with a single enantiomer of **2**, this problem may be overcome.

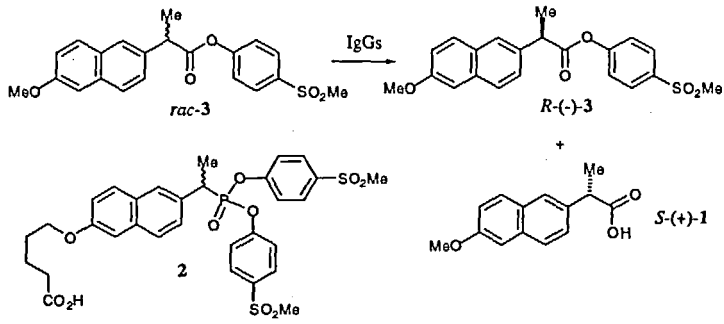

FIGURE 1 Phosphonate diester **2** was utilized as a hapten to elicit enantioselective catalysts for the hydrolysis of aryl esters **3** of naproxen **1**.

Chemical selection for catalysis from combinatorial antibody libraries

Whereas biological selection processes in antibody combinatorial libraries are based on survival, for chemistry we must only discriminate between those library members that can and cannot accomplish a chosen reaction. In phage systems this can simplified to either a binding event (non-covalent) or a covalent interaction to trap those library members that are catalytic. For this purpose, we have developed a process dubbed 'chemical selection' whereby catalytic Fab-phage complexes are highlighted from combinatorial libraries using a mechanism-based screen[8,9].

Glycosidic bond cleavage[9]

Hydrolysis of the glycosidic bond in the immobilized reagent **4** generates a reactive quinone methide **5** which may covalently trap any Fabs in the test library capable of catalyzing its formation (see Figure 2). A Fab, 1B, isolated by this methodology, catalyzes the hydrolysis of *p*-nitrophenyl β-galactopyranoside with an enhancement ratio (ER) of 70,000, comparing very favorably with the best antibody generated by a classical TSA approach (ER of only 100).

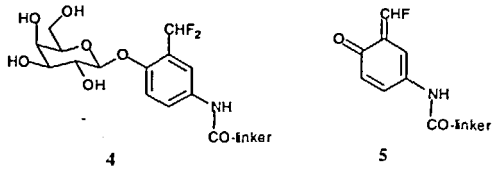

FIGURE 2 Reagent **4** was utilized in a chemical selection strategy to isolate Fabs which catalyze the hydrolysis of glycosidic bonds. The quinone methide **5** traps and thus highlights catalytic Fabs.

Peptide boronic acid haptens

Boronic acids are potent inhibitors of serine protease enzymes, their *modus operandi* being linked to coordination of the binding site serine and/or histidine residues to form tetrahedral anionic enzyme-bound intermediates[10-12].

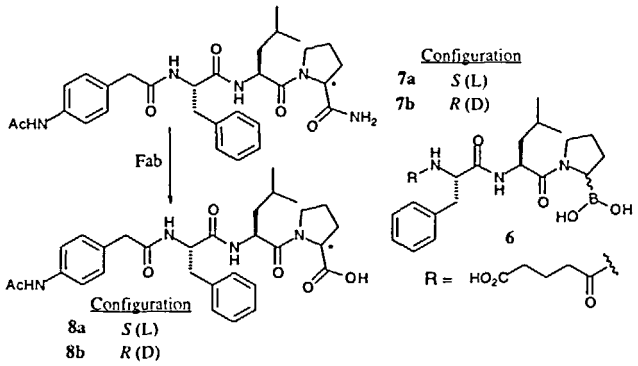

FIGURE 3 Peptide boronic acid **6** was used as a panning agent to select for Fabs that could accelerate the hydrolysis of primary amide **7a,b** to the free acid **8a,b**.

In an attempt to generate proficient antibody catalysts for amide bond hydrolysis, the α-aminoboronic acid hapten **6** was employed in a chemical selection strategy (see Figure 3)[13]. The boronic acid moiety can serve both as a TSA (see Figure 4A) and as a trap for Lewis base residues in the antibody binding pockets (see Figure 4B). A phage-Fab library (*ca.* 2 x 10^8 members) was panned at pH 7.5, at which pH the effective concentrations of the trigonal and tetrahedral forms of **6** are expected to be equivalent[10]. The adherent phage were eluted with an acidic wash (0.1 M HCl, pH 2.2) which presumably either

perturbed the hydration equilibrium of **6** back to a trigonal form or directly hydrolyzed the boronate esters and amides formed through binding site Lewis base coordination to **6**.

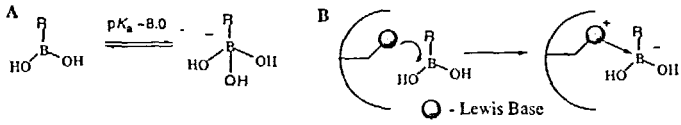

FIGURE 4 Mechanisms by which boronic acids may elicit acyl transfer catalysts:
A) The tetrahedral anionic hydrated boronic acid mimics the transition state
B) The trigonal acid can trap a Lewis base in the antibody combining site.

From the family of adherent phage-Fab, BL25 was chosen for further study based on its expression level. Fab-BL25 catalyzes the hydrolysis of the L-proline primary amide **7a** to its free acid **8a** (k_{cat} = 0.003 min^{-1}, K_m = 150 μM)[13], discriminating against its D-proline diastereoisomer **7b** which is not a substrate. The calculated ER (k_{cat}/k_{uncat}) of *ca.* 4 x 10^4 is > 2 orders of magnitude higher than that observed for an antibody elicited by the TSA approach[14], highlighting the power of this direct selection strategy.

Acknowledgments
This work was supported in part by the National Institutes of Health (NIH-GM 43858, KDJ) and the Skaggs Institute for Chemical Biology.

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